8 NORMALIZATION WIZARD AND AMPLIFICATION PROCESSES

8.1 GOALS:

8.1.1 To develop an understanding and working knowledge of the amplification process, including proper documentation.

8.1.2 To become familiar with problems associated with amplification.

8.1.3 To understand the importance of an amplifying environment that has no contamination.

8.1.4 To understand the importance of quality control associated with the amplification process.

8.2 TASKS:

8.2.1 Work in an environment free of contamination and follow proper guidelines to prevent contamination.

8.2.2 Program a thermal cycler and perform the quality control test on the thermal cycler, completing all appropriate documentation.

8.2.3 Perform manual amplification setup on half of the training samples addressed in Section 4, DNA Isolation, using the PowerPlex® 16 BIO System. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for the procedure.

8.2.4 Observe the Project Coordinator run the second half of the training samples addressed in Section 4, DNA Isolation, through the entire Normalization Wizard, amplification setup and deck setup using the BioMek® 2000 Automation Workstation.

8.2.5 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B, and C.

8.2.6 Continue on to Chapter 9, PRODUCT GEL

8.3 TRAINING EVALUATION:

8.3.1 Knowledge

8.3.1.1 Review of notes and worksheets in training notebook by training coordinator.

8.3.1.2 Mini-mock trials and/or question and answer sessions.

8.3.2 Skills

8.3.2.1 The trainee should demonstrate an unquestionably sound technique for DNA amplification by consistently achieving uncontaminated results on the product gel. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

8.3.3 Completion of trainee the checklist by training coordinator.

STUDY QUESTIONS:

1. What is a nuclease?
2. What is an endonuclease?
3. What is an exonuclease?
4. Explain the amplification process.
5. What is a DNA polymerase?
6. What is the name of the DNA polymerase that is used by the DFS and how does it work? Why do we use this DNA polymerase?
7. What is a primer?
8. What is the function of the primer?
9. What is the origin of the primer?
10. What is the function of $MgCl_2$?
11. What impact does the use of AmpliTaq Gold™ have on the PCR process?
12. What is primer - dimer?
13. How can primer - dimer affect the results?
14. Explain denaturation, annealing, and extension of the DNA.
15. What is preferential amplification (allelic drop out) and why does this occur? Is this a problem when analyzing samples using the STR technology? Why or why not?
16. What precautions are used to ensure that allelic drop out has not occurred?
17. What is plateau effect and how does it affect the DNA sample?
18. What are the components of the PowerPlex® 16 BIO reaction mix? What is the purpose of each component?
19. What are some of the factors that inhibit amplification and why? What steps can the analyst take to overcome inhibition problems?
20. What are the amplification conditions for the PowerPlex® 16 BIO System kit?

21. How many primers are used in the PowerPlex® 16 BIO System?

22. What precautions are used to prevent contamination of the sample DNA with a foreign source?

23. What measures are taken to ensure that the thermal cycler is working properly? What is the purpose of each quality control test?

CHECKLIST FOR NORMALIZATION WIZARD AND AMPLIFICATION PROCESSES

Name of Trainee: _____

1. The trainee has demonstrated he/she can work in an environment free of contamination and follow proper guidelines to prevent contamination.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee has successfully programmed and performed quality control testing on the thermal cycler and completed the appropriate documentation.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee has successfully and accurately completed all appropriate paperwork associated with the amplification of the samples using the PowerPlex® 16 BIO System.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee has manually performed amplification on the following samples using the PowerPlex® 16 BIO System:

   - A minimum of 14 blood stains extracted using the organic, manual DNA IQ™ and automated DNA IQ™ extraction methods.
   - A minimum of 14 unmixed body fluid stains, to include semen, vaginal fluid, saliva, vasectomized semen samples, urine and feces, using the organic, manual DNA IQ™ and automated DNA IQ™ extraction methods.
   - A minimum of 10 mixed biological stains (at total of 20 samples), to include semen, vaginal fluid, blood, and saliva using the differential organic, manual DNA IQ™ and automated DNA IQ™ extraction methods.
   - Half of the validation samples using either an organic extraction method, manual DNA IQ™ extraction method or the automated DNA IQ™ extraction method.
     - Two blood/bone/tissue sample sets, each set from a different individual
     - Five animal samples
     - Ten contaminated stains
     - Five blood/semen/hair sets
     - A family study
     - Non-probative case samples (at least 5 cases)

- Ten old stains (at least 5 years old)
- Two buccal swab/teeth sample sets

Date:_____ Training Coordinator:_____

Comments:_____

5. Trainee has observed the Project Coordinator run the second half of the training samples through the entire Normalization Wizard, amplification setup, and deck setup using the BioMek® 2000 Automation Workstation.

   Date:_____ Project Coordinator:_____

   Comments:_____

6. Trainee has developed an understanding of the theory of the amplification process, including the importance of quality control.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

8. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

9. Trainee has participated in mini-mock trials and/or question and answer sessions.

   Date:_____ Training Coordinator:_____

   Comments:_____

♦END